# CDPK5 AND MSP1 GENE MODULATION AND *IN VITRO* PARASITE INHIBITION BY MCL OR ITS COMBINATION WITH ARTEMISININ

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**AUGUST 2023**

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**BY**

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**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE, (M.Sc) IN BIOCHEMISTRY IN THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA, OGUN STATE, NIGERIA**

**AUGUST 2023**

# ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfilment of the requirements for the award of Master of Science (M.Sc.) degree in Biochemistry in the Department of Biological Sciences, College of Science and Technology, Covenant University Ota, Ogun State, Nigeria.

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**Prof. Akan B. Williams**

**(Dean, School of Postgraduate Studies)** **Signature and Date**

# DECLARATION

**I, OKAFOR, ESTHER OGECHI (21PCP02254)** declare that I carried out this research under the supervision of Dr. Titilope M. Dokunmu of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the dissertation has not been presented wholly or partially for the award of any degree elsewhere. All the sources of materials and scholarly publications used in the dissertation have been duly acknowledged.

**OKAFOR, ESTHER OGECHI**

**Signature and Date**

# CERTIFICATION

We certify that this dissertation titled **"CDPK5 AND MSP1 GENE MODULATION AND *In vitro* PARASITE INHIBITION BY MCL OR ITS COMBINATION WITH ARTEMISININ"** is an original work carried out by **OKAFOR, ESTHER OGECHI** (**21PCP02254**) in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Dr. Titilope M. Dokunmu. We have examined and found the work acceptable as part of the requirements for the award of a degree of Master of Science (M.Sc.) in Biochemistry.

**Dr Titilope M. Dokunmu**
**(Supervisor)** **Signature and Date**

**Prof. Israel S. Afolabi**
**(Head of Department)** **Signature and Date**

**Prof. Adedoyin Igunnu**
**(External Examiner)** **Signature and Date**

**Prof. Akan B. Williams**
**(Dean, School of Postgraduate Studies)** **Signature and Date**

# DEDICATION

This report is dedicated to the almighty God, the giver, and sustainer of life, for His unconditional love and mercy granted to me through this project. And to my parents Mr. and Mrs. Okafor and siblings for their constant love and support.

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# ABBREVIATIONS

| | |
|---|---|
| ART | artemisinin |
| BSA | Bovine Serum Albumin |
| CQ | chloroquine |
| DMSO | dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| HEPES | 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid |
| $IC_{50}$ | 50% inhibitory concentration |
| MSP2 | Merozoite Surface Protein 2 |
| MW | molecular weight |
| PCR | Polymerase chain reaction |
| *PfCRT* | *P. falciparum C*hloroquine esistance transporter gene |
| *Pf*MDR1 | *P. falciparum* multidrug resistance gene 1 |
| *Pf*MRP | *P. falciparum* multidrug resistance-associated protein |
| *Pf*MSP1 | Merozoite Surface Protein 1 |
| *Pf*NHEL | *Plasmodium falciparum* sodium hydrogen exchanger |
| RBC | red blood cell |
| RPMI | Roswell Park Memorial Institute |
| RSA | ring-stage survival assay |
| RT | room temperature |
| WBC | white blood cell |
| WHO | World Health Organization |

# ABSTRACT

Recent treatment failures in artemisinin-based combination therapy (ACT) have raised concerns about its efficacy against malaria and emphasize the need to discover new treatment targets and resistance-free drugs. A small molecule (MCULE-7146940834 - MCL) showed inhibitory potential against Plasmodium falciparum, targeting gene families crucial for red blood cell invasion in silico but has not been validated in vitro. This study assesses MCULE-7146940834 in vitro, evaluating its inhibitory concentration (IC50) and gene modulation effects on Merozoite Surface Protein 1 (*Pf*MSP1) and Calcium-dependent Protein Kinase (*Pf*CDPK5), both independently and in combination with Artemisinin (ART). *Pf*MSP1 facilitates the attachment and binding of the merozoite to the host RBC, while *Pf*CDPK5, facilitates the secretion of invasion-related proteins and motor function to drive penetration making them promising antimalarial drug targets. *Plasmodium falciparum* derived from field isolates and the 3D7 strain were cultured within O+ human red blood cells. This cultivation occurred in RPMI 1640 medium supplemented with 10% heat-inactivated human serum, 25 mM HEPES buffer, and 50 µg/ml penicillin-streptomycin. The entire process was carried out under controlled conditions of 5% CO2 at 37°C. Serially diluted drugs of ART, ART+MCL, and MCL were administered to 96-well microtitre plates, over 72 hours, with doses incrementing by a factor of 10 from 0 to 100µM. This procedure adhered to the WHO micro-test protocol and involved incubating the substances with parasite culture medium samples at a parasitemia level of 0.2% and a haematocrit of 4% for the same duration. The evaluation of parasitemia was performed microscopically using Giemsa-stained smears. RNA from cultured samples, pre- and post-treatment, was extracted, quantified, and analysed by real-time polymerase chain reaction using primers specific for the *Pf*MSP1 and *Pf*CDPK5 genes and *Pf*GAPDH as an internal reference gene. All assays were carried out in duplicates and analyzed using graph pad prism software at $p < 0.05$. The outcomes from the analysis of half maximal inhibitory concentrations (IC50) using linear regression demonstrated an *in vitro* $IC_{50}$ value of 24.68 µM for MCL, and a value of 5.006 µM for the combination of MCL with artemisinin. Relative gene expression (ΔCt) shows increased expression of *Pf*MSP1 and *Pf*CDPK5 relative to *Pf*GAPDH. These results demonstrated that the MCULE-7146940834 holds promise as a potential candidate for antimalarial drug development, making it a valuable hit compound for subsequent optimization.